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Minireview

Variation in proton donor/acceptor pathways in succinate:quinone oxidoreductases

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Abstract The anaerobically expressed fumarate reductase and aerobically expressed succinate dehydrogenase from *Escherichia coli* comprise two different classes of succinate:quinone oxidoreductases (SQR), often termed respiratory complex II. The X-ray structures of both membrane-bound complexes have revealed that while the catalytic/soluble domains are structurally similar the quinone binding domains of the enzyme complexes are significantly different. These results suggest that the anaerobic and aerobic forms of complex II have evolved different mechanisms for electron and proton transfer in their respective membrane domains.

Key words: Succinate dehydrogenase; Fumarate reductase; Quinone oxidoreductase; Complex II; Electron transport; Proton

1. Introduction

Complex II refers to a family of enzymes comprising succinate:quinone oxidoreductases (SQR) and the structurally and functionally related quinol:fumarate oxidoreductases (QFR). Complex II serves a dual function within the cell as part of the respiratory electron transport chain and also as a component of the citric acid cycle where it oxidizes succinate to fumarate. During the oxidation of succinate to fumarate by SQR two electrons and two protons are generated and the covalently bound flavin cofactor of the enzyme is reduced. The electrons from the flavin are transferred sequentially through a series of three iron–sulfur clusters to ubiquinone (UQ) in the membrane domain for entry into the electron transport chain and two protons are used for reduction of UQ. In the case of *Escherichia coli* SQR there is no net generation of a transmembrane proton gradient during catalysis as the protons generated by succinate oxidation and those used for quinone reduction remain on the cytoplasmic side of the membrane. As the only membrane-bound component of the citric acid cycle, SQR is uniquely positioned to regulate the formation of reducing equivalents during metabolism.

This article will focus on the SQR from *E. coli* which by all criteria would be classed with mammalian mitochondrial SQR and *E. coli* QFR. The latter is of the class of complex II that does not contain a *b*-type heme [1–3]. SQR and QFR from *E. coli* are each composed of four subunits with a total molecular mass of approximately 120 kDa (Fig. 1A,C). There is a hydrophilic domain composed of the flavoprotein (SdhA, FrdA) and iron–sulfur protein (SdhB, FrdB) subunits. The flavoprotein subunit contains a covalently bound FAD cofactor as part of the catalytic site [4,5] which is remarkably conserved amongst both SQR and QFR [6]. There are three distinct linearly arranged clusters in the iron–sulfur subunit, a $[2Fe–2S]^{2+,1+}$, $[4Fe–4S]^{2+,1+}$, and $[3Fe–4S]^{1+,0}$ cluster, which transfer electrons between the FAD cofactor and quinones in the membrane domain. The hydrophilic domain is bound to the membrane domain through interactions between the iron–sulfur protein and the two hydrophobic subunits (SdhC/SdhD, FrdC/FrdD). There is significant sequence variation amongst the subunits of the hydrophobic domain of complex II. The SQR family of enzymes has been classified into three to five separate groups with a variety of criteria used for classification. These include the type of quinone used as acceptor/donor, the number of *b*-type hemes associated with the enzyme, and the number of hydrophobic membrane anchor subunits in the complex [1–3]. Nevertheless, the available X-ray structures [6–8] and models for complex II [9] suggest that the transmembrane helices are arranged around a four-helix bundle with two helices coming from each membrane anchor subunit. In both the *E. coli* SQR and QFR the two transmembrane helices lying outside the helical core encompass the C-terminal portion of each anchor subunit (Fig. 1B,D). In both the C and D subunits of QFR/SQR the NH_2-terminus is on the cytoplasmic side of the membrane (matrix side in mitochondria), whereas the carboxy-terminus is on the periplasmic side of the membrane. Nevertheless, major structural differences are found in the transmembrane domain of the two *E. coli* homologs of complex II. One differ-

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Abbreviations: DNP-19, 2-[1-*p*-chlorophenyl)ethyl]-4,6-dinitrophenol; HQNO, 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide; MQ, menaquinone; Q_D, quinone distal binding site; Q_P, quinone proximal binding site; QFR, quinol:fumarate oxidoreductase; SQR, succinate:quinone oxidoreductase; UQ, ubiquinone

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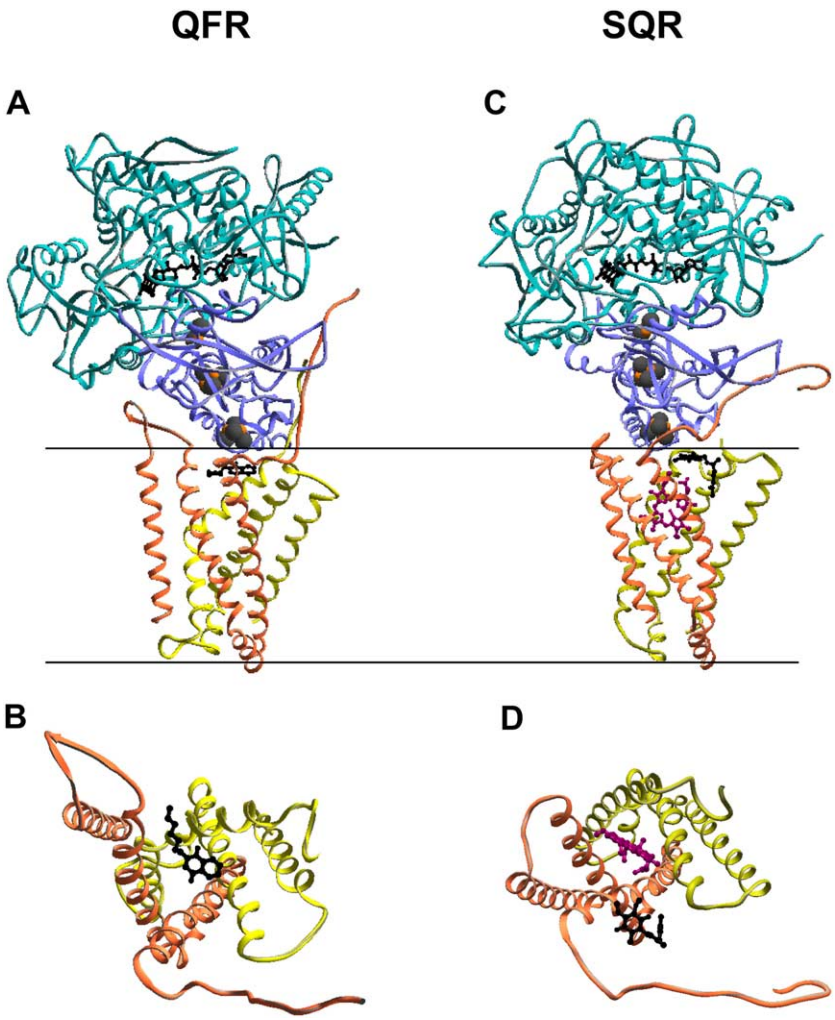

Fig. 1. Three-dimensional structures of *E. coli* QFR and SQR. The QFR structure (1KF6) is shown with the inhibitor HQNO in the proximal quinone binding site. SQR is drawn from PDB coordinate set 1NEK with UQ in the quinone binding site. The dehydrogenase domains consisting of subunits A and B have the Cα traces colored teal and purple, respectively. The Cα traces of the two transmembrane subunits C and D are shown in orange and yellow, respectively. Overview of QFR (A) and SQR (C) structures. The prosthetic groups are from top to bottom: covalently bound FAD, the [2Fe–2S], the [4Fe–4S], and [3Fe–4S] iron–sulfur centers. The proximal quinone binding site is occupied by HQNO in QFR and UQ in SQR. The heme b_{556} in SQR is shown in magenta (C,D). A view of subunits C and D (panels B and D) perpendicular to the membrane highlights the four-helix bundle adopted by the membrane-spanning regions of these two enzymes. B: Subunits FrdC and FrdD are shown with the bound inhibitor HQNO. D: Subunits SdhC and SdhD are coordinated to the heme b_{556} and have UQ in the quinone binding site. The figure was made using Swiss-pdbViewer 3.7.

ence is the lack of a *b* heme moiety in the QFR complex from *E. coli* (compare Fig. 1A vs. 1C). Other differences include the position of the quinone binding site, amino acid residues used to stabilize the bound quinone species, and the relative orientation of the four-helix bundle with respect to the soluble domain [6]. The structural difference in the quinone binding sites and amino acid residues used for protonation/deprotonation reactions with quinones is the focus of this article as discussed below.

2. Catalytic reactions of QFR and SQR

Although both QFR and SQR can be poised to catalyze the same reactions in vitro and in vivo, in the cell their physiological reactions are different [10]. QFR is part of an anaerobic electron transport chain where oxidative phosphorylation is coupled to the respiratory chain by membrane-bound dehydrogenases. In the *E. coli* QFR, the low potential menaquinone (MQ; $E_m = -74$ mV) is oxidized in the membrane domain near the [3Fe–4S] cluster on the cytoplasmic side of the membrane followed by reduction of fumarate in the cytoplasm. During aerobic metabolism succinate is oxidized by SQR in the cytoplasm (or matrix in the mitochondrion) and electrons are donated to UQ) $E_m = +90$ mV) in the membrane domain generating ubiquinol which is used by other components of the electron transport chain. As eloquently discussed previously [2] the variety of complex IIs from different organisms appear to have evolved different ways for the movement of protons and electrons in the membrane domain during succinate oxidation and fumarate reduction. It is generally assumed that SQR from mitochondria and *E. coli* are not involved in production of a transmembrane proton gradient (see Fig. 2A,B for models). As noted, the electron transfer reaction in *E. coli* SQR is energetically favorable since the

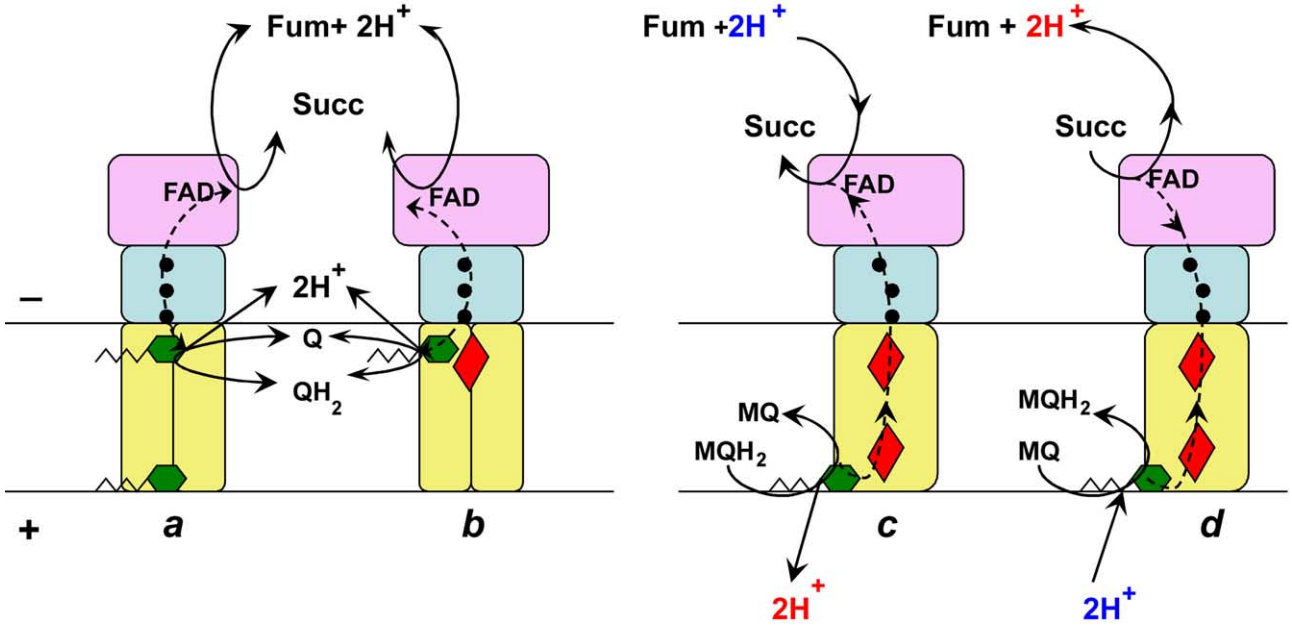

Fig. 2. Coupling of proton and electron flow in *E. coli* and *B. subtilis* complex II. a: *E. coli* QFR. b: *E. coli* SQR. c: *B. subtilis* SQR functioning in fumarate respiration as a menaquinol oxidase. d: *B. subtilis* SQR functioning in its normal physiological reaction as a succino-oxidase and MQ reductase. The positive side of the membrane (periplasmic side in the case of *E. coli*) is indicated by +. The negative side of the membrane is indicated by −. The reactions catalyzed by *E. coli* QFR and SQR shown in a and b are electroneutral and do not generate a net proton gradient. In c the net generation of a proton gradient by *B. subtilis* SQR is indicated, whereas in d the utilization of the electrochemical potential is indicated. In the figure the dark closed circles indicate the Fe–S clusters of the enzymes; the green hexagons indicate the position of quinone molecules; the red diamonds indicate the number and positions of the *b* hemes.

succinate/fumarate couple has a lower E_m (+25 mV) than the acceptor UQ (+90 mV) [2] and experimental data show that electron transfer is not sensitive to uncouplers [11].

By contrast, the di-heme SQR from *Bacillus subtilis* uses low potential MQ as electron acceptor (Fig. 2C,D) [2,12]. The succinate to MQ oxidoreductase reaction is strongly endergonic and sensitive to uncouplers and agents that dissipate the membrane potential (Fig. 2D) [11]. Protonation of MQ in *B. subtilis* is thought to occur on the outside of the membrane (equivalent to the periplasmic side in *E. coli*) [13]. In *B. subtilis* it has been experimentally verified that fumarate reduction from menaquinol coupled to NADH oxidation generates a protonmotive force (Fig. 2D) [12]. The topology of the *B. subtilis* SQR, with the site of menaquinol oxidation on the outside of the membrane [14], leads to the suggestion that a proton potential is generated by fumarate reduction (Fig. 2C) [12]. Thus, a mechanism exists whereby H^+ from the cytoplasm used in MQ reduction is released to the outside face of the membrane by menaquinol oxidation [12] in *B. subtilis* SQR. It has been speculated that the *Wolinella succinogenes* QFR could also induce proton transfer by a type of Mitchellian Q loop with a net proton electrochemical gradient being formed [2]. The structure of the *W. succinogenes* QFR has been solved to 2.2 Å resolution [8] and like *B. subtilis* SQR it is a complex II containing two *b*-type hemes and a single transmembrane hydrophobic subunit [1,3]. However, experimental results for *W. succinogenes* QFR using liposomes and intact bacteria suggest that fumarate-dependent oxidation of menaquinol catalyzed by QFR is an electroneutral process [15,16] unlike what is found in *B. subtilis* SQR [12]. This seems somewhat surprising in light of the finding that a residue in the membrane domain C-subunit (C Glu66) is essential for menaquinol oxidation [17] and the spatial location of this residue demonstrates that it is on the periplasmic side of the membrane. This structural arrangement of the menaquinol oxidation site would suggest that protons could be released into the periplasm (similar to what is shown in Fig. 2C for *B. subtilis* SQR) although this is not consistent with the available data for *W. succinogenes* QFR [15,16].

In order to rationalize these apparent differences between *B. subtilis* SQR and *W. succinogenes* QFR, a suggestion was recently made termed the 'E-pathway hypothesis' [18]. In this proposed mechanism protons liberated at the menaquinol oxidation site, rather than being liberated into the periplasm of *W. succinogenes*, are transferred through the membrane domain of QFR through a proton transfer pathway involving the heme propionates and transmembrane helix V of *W. succinogenes* QFR (not shown) [18]. The net effect is that the protons remain on the cytoplasmic side of the membrane so that no transmembrane electrochemical potential is generated. Although this contention is attractive in rationalizing the available data it remains to be proven. The differences in potential pathways for movement of protons in *B. subtilis* and *W. succinogenes* complex II, nevertheless, highlight the fact that nature seems to have evolved unique mechanisms in what on the surface seem highly similar enzymes.

3. Distal quinone binding site (Q_D) of *E. coli* QFR

In light of the proposals for the quinol oxidation site of *B. subtilis* SQR and *W. succinogenes* QFR, the role of the Q_D site in *E. coli* QFR remains enigmatic. In both the *E. coli* SQR [6] and QFR [7] X-ray structures, quinone molecules are found in the membrane-spanning region. In the case of *E. coli* QFR there are two MQ molecules located on opposite sides of the transmembrane domain [7] (see Fig. 2A). The

edge-to-edge separation of these MQ molecules is ~25 Å which would suggest that they cannot participate as a pair in electron transfer reactions because of the large spatial separation [2,19]. It has been pointed out that electron transfer between the proximal quinone (Q_P) and Q_D would require hours for direct electron tunneling [2] unless there was an intervening redox cofactor present to shorten the tunneling distance. In contrast to the *W. succinogenes* QFR structure where a glutamate residue is found at the site for menaquinol oxidation [17] the Q_D site for *E. coli* QFR is a relatively hydrophobic pocket [7]. The apolar nature of the Q_D site resembles that found in the Q_A site of photosynthetic reaction centers [20] although the Q_D site does not contain any ionizable groups or H-bond acceptors near the O1 or O4 atoms of MQ. In the photosynthetic reaction centers the quinone at Q_A can only be singly reduced to the semiquinone (Q_A^-) thus only oxidized or semiquinone states are found at the Q_A site. This has been suggested to result from the inaccessibility of the Q_A site to protons [20]. Thus, the hydrophobic nature of the Q_D site of *E. coli* QFR seems to argue against its involvement in proton translocation and its distance from any other known redox cofactor in the QFR structure also seems to preclude involvement in electron transfer.

It is pertinent therefore that in a recent higher resolution structure of *E. coli* QFR (2.7 Å) done in the presence of the quinol binding site inhibitors 2-heptyl-4-hydroxyquinoline-*N*-oxide (HQNO) or 2-[1-*p*-chlorophenyl)ethyl]4,6-dinitrophenol (DNP-19) no density for quinone at the Q_D site is observed [21]. It is possible that like for the bc_1 complex binding of the inhibitors reduces the affinity for the quinone at the second Q binding site in the bc_1 dimer [22] as a result of anti-cooperative binding behavior. This suggestion is reflected by the fact that in the original native structure [7] the density at Q_D is stronger than the density at Q_P. The structural data do suggest, however, that the presence of a quinone at the Q_D site is not a necessary prerequisite for protein stability [21] as the enzyme complex is stable even in the absence of the second quinone. It is also pertinent that in the *E. coli* SQR structure which is now available [6] no density for quinone is found in the structure at a site on the periplasmic side of the membrane equivalent to Q_D, although there is a UQ binding site proximal to the [3Fe–4S] cluster on the cytoplasmic side of the membrane.

A role for a quinone at the Q_D site in *E. coli* QFR cannot, however, be absolutely dismissed because of available site-directed mutagenesis data for both *E. coli* QFR [23] and *Saccharomyces cerevisiae* SQR [24]. In both of these complex IIs, mutation of amino acid residues in the vicinity of the Q_D binding site does affect the activity of the complex with quinones. There is, however, only a two-fold reduction in activity, suggesting that this loss could reflect minor structural alterations in the protein complex. Since the enzyme complex still assembles even in the presence of these mutations [23,24], it appears most likely that subtle alterations in the membrane domain might have effects at the Q_P site which would result in the lowered activity observed.

4. The proximal quinone binding sites (Q_P) for QFR and SQR

4.1. QFR Q_P site

It has been determined from biochemical measurements that there is a single exchangeable quinone binding site where oxidation of quinol occurs in *E. coli* QFR [25,26]. The site for menaquinol oxidation in the *E. coli* QFR complex can be inferred from the structural data as well as extensive biochemical and electron paramagnetic resonance (EPR) analysis of wild-type and mutant forms of the enzyme. In *E. coli* QFR, a MQ molecule termed Q_P is found at the interface of the membrane domain and the soluble domain of the four-subunit complex (Fig. 3A). The fully reduced menaquinol at the Q_P binding site must release two electrons and two protons following oxidation to quinone. The Q_P quinone is 8–9 Å from the [3Fe–4S] cluster found in the iron–sulfur subunit FrdB. As *E. coli* QFR functions bi-directionally as a succinate-quinone reductase or as a quinol-fumarate oxidase it is apparent that the [3Fe–4S] cluster is the direct donor or acceptor of the electrons for quinone reduction or quinol oxidation.

The proton transfer path for these two processes, however, appears to be different. Amino acids in close proximity to the quinone at the Q_P binding site are shown in Fig. 3A. Two hydrogen bond donors, Lys-B228 and Trp-D14, are positioned within hydrogen bonding distance of the C4 carbonyl oxygen atom of MQ (Fig. 3B). As pointed out previously, hydrogen bonds from these residues would satisfy the hydrogen bonding capabilities for MQ or a menasemiquinone [21]. The C1 carbonyl oxygen atom of the MQ head group is within hydrogen bonding distance of Glu-C29 and Arg-D81 side chains (Fig. 3C). It can also be seen in Fig. 3 that additional residues such as Gln-B225, Arg-C28, and Cys-204, which is one of the ligands of the [3Fe–4S] cluster, are also in close spatial proximity to this site.

The semiquinone generated during oxidation of quinol at the Q_P site must be at least transiently stabilized as the [3Fe–4S] cluster is a one-electron acceptor. Thus, it is relevant that EPR analysis has shown that mutation of Glu-C29 to a neutral amino acid residue results in a semiquinone at the Q_P site with a significantly raised stability constant of $\sim 1.2\times10^{-2}$ [27]. It was also found in these studies that in wild-type QFR the stabilized semiquinone has a stability constant some four orders of magnitude lower than in mutant enzyme. Thus, in wild-type QFR it is likely that there is a thermodynamically relatively unstable semiquinone at the Q_P site which is in line with its function as a quinol oxidase [27]. In the cytochrome bc_1 complex Glu-272 of the cytochrome *b* subunit has been suggested to be involved in proton shuttling rather than in stabilization of a protonated quinone species [28]. Glu-C29 in *E. coli* QFR, as stated above, does not stabilize the semiquinone per se and its role thus may be analogous to the suggested role for Glu-272 of the bc_1 complex. It had previously been suggested that the role of Glu-C29 in *E. coli* QFR was to act as an acceptor/donor for protons during quinone oxidation/reduction [23]. The available structural [21], biochemical [23], and EPR [27] analyses are all consistent with its role as acceptor of protons during quinol oxidation, however, the mutagenic studies suggest that it is not necessary for it to act as a proton donor.

As quinones have two oxygen atoms on opposite sides of the molecule, each must be protonated or deprotonated during reduction/oxidation to go from oxidized to reduced quinone, respectively. It is believed that the protons used for this process move along the side chains of protonatable amino acids from the protein backbone or from water molecules. The presence of an acidic residue at an exchangeable quinone binding site is found in a number of respiratory proteins [21]

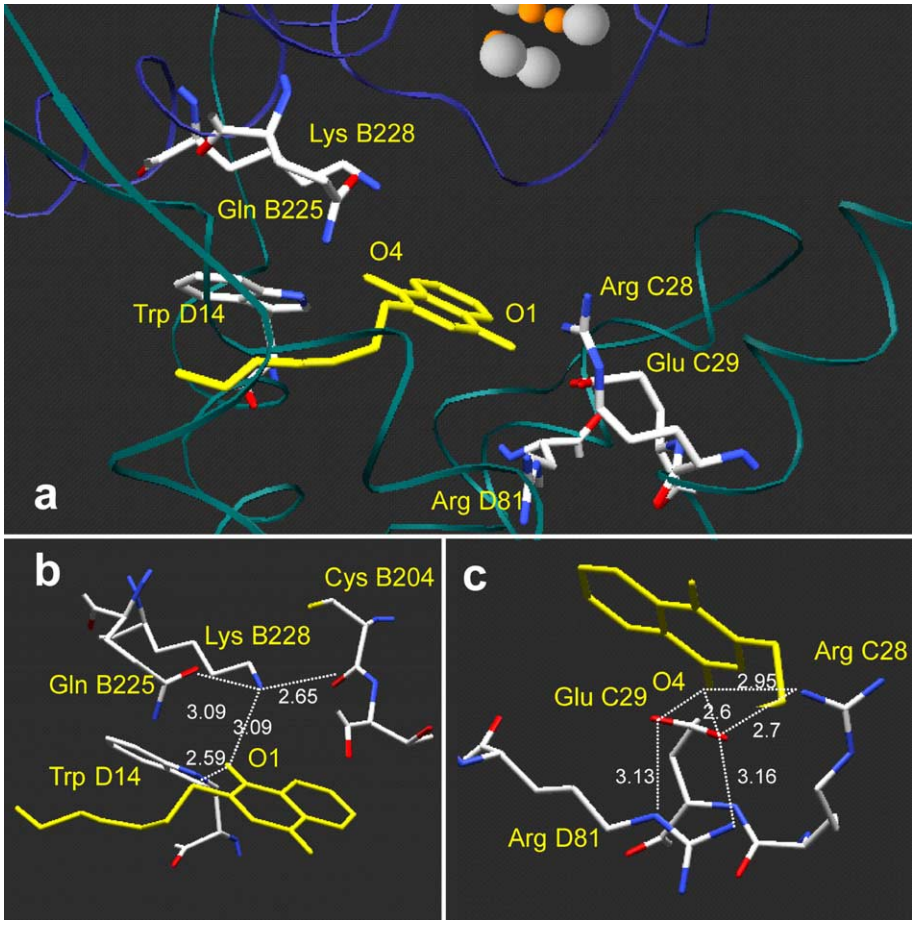

Fig. 3. The proximal (Q_P) quinone binding site of *E. coli* QFR. a: Cα traces of FrdB are colored dark blue, while those of FrdC and FrdD are green. The side chain position of residues proximate to oxygen in the MQ molecule are taken from the structure of QFR with HQNO bound (1KF6, 2.7 Å resolution) and are within the error of the side chain positions from the structure of QFR with MQ (1L0V, 3.3 Å resolution). Oxygen atoms are colored red and nitrogen are blue. The HQNO inhibitor is colored yellow. Iron and sulfur atoms of the Fe–S center [3Fe–4S] are orange and gray respectively. The QFR residues within proximity to C_4 hydroxyl (b) and C_1 hydroxyl (c) of the menaquinol moiety are shown. Dashed lines indicate the hydrogen bonding interactions with distances between atoms indicated. The figure was made using Swiss-pdbViewer 3.7.

including the *W. succinogenes* QFR Glu-C66 [17] and the *B. subtilis* SQR Asp-C52 [29]. In the case of *E. coli* QFR it certainly seems that Glu-C29 plays a role as a proton shuttle during menaquinol oxidation. It is important to note that assay of the catalytic activity of QFR Glu-C29 mutants shows that catalytic activity with MQ is reduced to about 2% of wild-type enzyme, whereas UQ reductase activity by the mutant remains unaffected (Maklashina and Cecchini, unpublished data). It is likely that the hydroxyl group on the opposite face of the quinone molecule is deprotonated during quinol oxidation by a chain of residues including Lys-B228 which is in proximity to this region of the enzyme Fig. 3B. [21].

It would appear reasonable to suggest from the structure shown in Fig. 3 that for the opposite reaction (UQ reduction), which is proficiently catalyzed by *E. coli* QFR, protons used for reduction of UQ are provided from amino acid residues such as Lys-B228 to the C4 carbonyl oxygen of the quinone. Since mutation of Glu-C29 has no effect on quinone reduction by *E. coli* QFR the direct donor of protons to the C1 carbonyl oxygen may involve a chain of residues and water molecules such as Arg-C28 or Arg-D81. It should be remembered that UQ is not the normal quinone utilized by QFR for catalysis, however, the enzyme is proficient in using either UQ or MQ. As the benzoquinone head group of UQ is somewhat smaller than the naphthoquinone head group of MQ it is conceivable that UQ occupies a somewhat different position in the Q_P binding pocket. For example, in *E. coli* QFR it has been shown that the inhibitor DNP-19 makes only hydrogen bonding contacts with one side of the quinol binding pocket, whereas HQNO which is a MQ analog has contacts on both sides as shown in Fig. 4 [21]. In the DNP-19 structure Lys-B228 and Trp-D14 make hydrogen bond contacts with the inhibitor, whereas Glu-C29 and Arg-D81 from the other side of the binding pocket do not make contact. The side chains of Gln-B225, Arg-C28, and Leu-C89 have moved in the QFR structure with DNP-19 in the Q_P site, consistent with the idea that different quinone species could occupy different parts of this site. It has been shown in the photosynthetic reaction center that quinones can show significant movement during reduction [30] and adopt different locations within the Q_B site.

In *E. coli* QFR a bifurcated pathway may exist for quinol oxidation or quinone reduction which would bring the arginine residues from one side of the Q_P pocket in closer contact with the oxygen atom of the quinone. Thus one can envision a

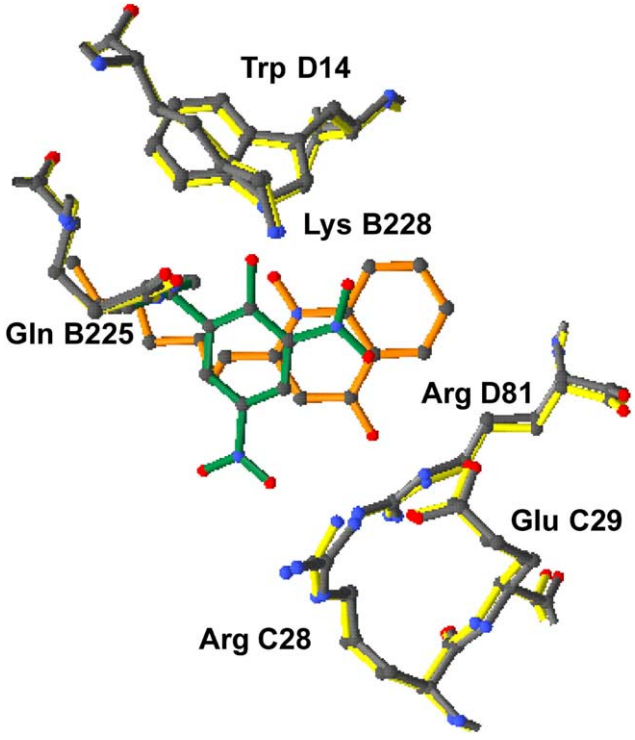

Fig. 4. Proximal quinone binding site of *E. coli* QFR occupied with HQNO and DNP-19. Residues from HQNO-bound enzyme (1KF6) have bonds between atoms colored yellow and residues from DNP-19-bound structure (1KFY) have the bonds between atoms in gray. The inhibitor HQNO is shown in orange, whereas the inhibitor DNP is colored green. Oxygen atoms are colored red, nitrogens blue, and carbon atoms are gray balls. The figure was made using Swiss-pdbViewer 3.7.

mechanism for QFR by which Lys-B228 and Glu-C29 are the immediate proton acceptors in the protein during quinol oxidation. During quinone reduction, however, a pathway exists whereby protons are donated from Lys-B228 to one oxygen atom, whereas the second proton comes from a pathway involving Arg-C28 or Arg-D81 and associated water molecules.

4.2. SQR quinone binding site

The recent solution of the X-ray structure of *E. coli* SQR at 2.6 Å resolution [6] has given us for the first time an opportunity to examine the UQ binding site in the family of complex II enzymes. A similarity for the *E. coli* SQR and QFR structures is that electron density assigned as quinones is found in the crystal structures [6,7]. In the case of SQR, however, only a single UQ binding site was found. Although there is little overall sequence conservation in the membrane domain subunits from various complex IIs it was nevertheless surprising to find that the UQ binding site was quite different from Q_P in the *E. coli* QFR [6]. The hydrophilic subunits of SQR can be superimposed with those from the available QFR structures. By contrast if the hydrophilic subunits are overlaid then the SQR UQ binding site is some 15 Å distant from the corresponding MQ Q_P binding site in QFR (Fig. 1B,D) [6]. When the hydrophilic subunits are overlaid from the two *E. coli* complex II structures the site equivalent to Q_P in QFR is occupied by the propionates from the single *b* heme which is found in SQR but not QFR [6]. Nevertheless, it is still relevant to term the site in SQR the Q_P binding site since it is proximal to the [3Fe–4S] cluster of the SdhB subunit. The [3Fe–4S] cluster is the closest redox center to the quinone (7.6 Å edge to edge), whereas it is some 9.8 Å from the *b* heme.

Fig. 5 shows residues forming part of the UQ binding site of SQR. Like those found in QFR [7,21] the amino acid residues from the iron–sulfur subunit and both transmem-

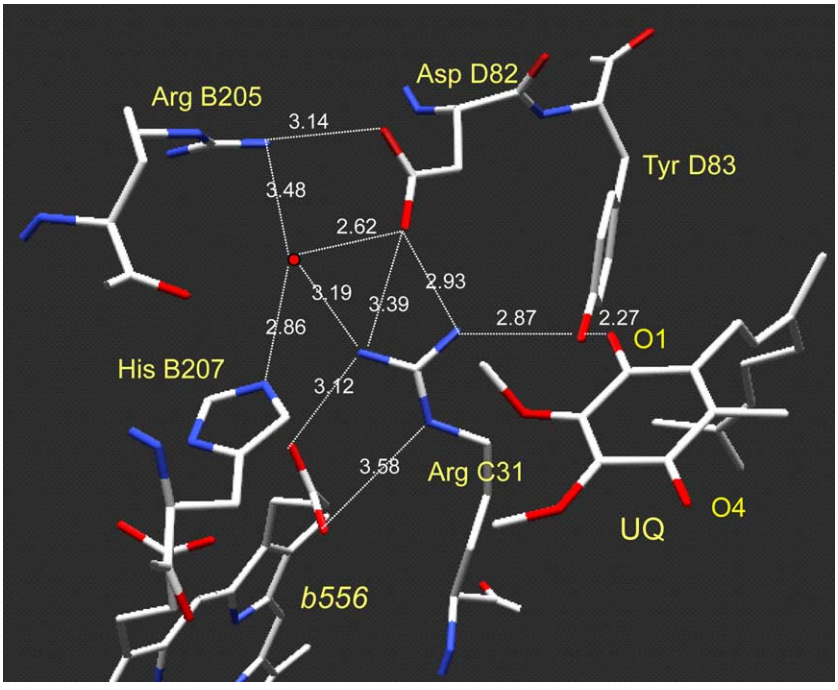

Fig. 5. Side chains surrounding the quinone binding site (Q_P) of *E. coli* SQR. The positions of the side chain residues, heme b_{556} and UQ are taken from the SQR structure (1NEK). The carbon atoms are colored white, oxygen and nitrogen are in red and blue, respectively. A water molecule is shown as a red dot. Dashed lines indicate the hydrogen bonding interactions with distances between atoms indicated on the figure. The figure was made using Swiss-pdbViewer 3.7.

brane anchor subunits form this binding site. Overall, the amino acid residues forming contacts with the quinone in the binding site in SQR are conserved in mammalian, *Caenorhabditis elegans*, *Ascaris suum*, *Paracoccus denitrificans*, *S. cerevisiae*, and *E. coli* SQRs, which suggests that they all belong to the same family. A direct ligand of the O1 carbonyl of UQ is Tyr-D83 which is oriented by a second hydrogen bond to Arg-C31. It has been suggested that this hydrogen bonding interaction would reduce the pK_a of the Tyr-D83 side chain [6]. Thus it appears likely that a proton may be directly transferred from Tyr-D83 to the O1 atom of UQ. *E. coli* SQR may be unique in this respect as to date there are no other examples of a tyrosine side chain being a direct H-bond donor for a quinone. Although there is not a large degree of sequence conservation in the membrane domain subunits of complex IIs the Tyr-D83 equivalent is conserved in all mammalian SQR sequences, suggesting its importance for function. In addition to its role as a proton donor, Tyr-D83 is likely to be necessary for stabilization of the anionic semiquinone, which has been identified in SQR [31,32].

If, like in the photosynthetic reaction center, the relative affinity of quinone for this binding site is anionic semiquinone > quinone > quinol [30] the electronic environment provided by Tyr-D83, Arg-C31, and Asp-D82 may be required for the highly stable semiquinone anion found in SQR [32]. If, as suggested above, the primary proton donor for the O1 carbonyl of UQ is Tyr-D83 it is still necessary to obtain a second proton to protonate the O4 carbonyl oxygen. As seen in Fig. 5, there is an absence of protein side chain in the vicinity of this atom. As this atom is close to the cytoplasmic surface of SQR it may be connected to the cytoplasm by a chain of water molecules which could be the potential proton donor. It should be noted that the Q_P binding site of SQR is more hydrophilic than the corresponding site in QFR. This is in agreement with studies that showed that inhibitors with increased hydrophobicity were stronger inhibitors of QFR than SQR [33]. The spatial arrangement of the binding site suggests that it might be possible that the O4 carbonyl oxygen is protonated first during quinone reduction by SQR. As stated above the semiquinone anion is the species most tightly associated with the enzyme [32]; thus if the O4 oxygen is protonated first the resultant anionic character of the oxygen at the O1 carbonyl could be stabilized by interaction with Tyr-D83. Following proton transfer to the O1 carbonyl oxygen from Tyr-D83 the ubiquinol generated would dissociate from the binding site and then be replaced by UQ to begin the next reaction cycle.

In Fig. 5, other residues that contribute to the overall topography of the UQ binding site are shown. The affinity for quinones for SQR from *P. denitrificans* [34] and bovine SQR [35] is greater than for the *E. coli* enzyme with K_D values one order of magnitude less, suggesting that the quinone binding site may be somewhat more hydrophobic in those enzymes. Data from *P. denitrificans* SQR have indicated that mutation of the residues equivalent to Asp-D82 and His-B207 confer resistance to carboxin which is a potent and specific inhibitor of the quinone reactions of SQR [34] but not of the *E. coli* QFR. As seen in the figure, there is a water molecule located approximately equidistant between Asp-D82 and His-B207. It would appear from the SQR structure that if carboxin were bound by the aspartate and histidine residues, it would displace the water molecule and disrupt the hydrogen bonding interactions near the Q_P site. If bound by these amino acid residues, carboxin would also sterically interfere with electron transfer from the [3Fe–4S] cluster to the quinone. The role of Arg-C31 is also of interest as in addition to possibly affecting the pK_a of Tyr-D83 it forms a salt bridge to a propionate of the b_{556} heme. It is known that when SQR is reduced there is a spectral shift in the *b* heme [36]. Thus it may be plausible to propose that reduction of the heme and UQ causes movement of Arg-C31 which also affects the electronic properties of the heme.

In summary, although *E. coli* SQR and QFR are both members of the complex II family of enzymes, the membrane-spanning domain including the Q_P quinone binding site appears to have evolved quite differently, which might reflect their different roles in aerobic and anaerobic metabolism [6]. Deprotonation of menaquinol in QFR seems to require an acidic residue like Glu-C29 similar to many other respiratory proteins containing exchangeable quinone binding sites [21]. In contrast, the UQ binding site in SQR appears rather unusual in that the most likely donor of the second proton to the anionic semiquinone is an aromatic tyrosine residue. The recent availability of the structure of SQR [6] will allow a more mechanistic study of the pathways for proton and electron transfer in this enzyme which should help elucidate interactions between the UQ and the *b* heme whose role remains enigmatic.

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